

Amendment and Reply

Atty. Dkt. No. P-5838

Serial No.: 10/660,760

Filing Date: September 12, 2003

Title: Methods of Surface Modification of a Flexible Substrate to Enhance Cell Adhesion

**AMENDMENTS TO THE CLAIMS**

**Please amend the claims with the according to the following claim listing.**

1. (Currently Amended) A method for producing a surface with enhanced cell-adhesive properties, comprising
  - a. applying a stress to a flexible polymeric matrix;
  - b. maintaining said flexible polymeric matrix as a strained matrix;
  - c. modifying the surface of said strained matrix by grafting a self-assembled monolayer onto said strained matrix, said self-assembled monolayer comprising at least one exposed functional group; and
  - d. activating said at least one functional group of said self-assembled monolayer; and
  - e. coupling at least one cell-adhesive molecule to said at least one active intermediate activated functional group on said self-assembled monolayer.
2. (Original) The method of claim 1, wherein said strained flexible polymer matrix is released after said self-assembled monolayer becomes grafted on the surface and prior to the addition of said at least one cell-adhesive molecule.
3. (Currently Amended) The method of claim 1, wherein said strained flexible polymer matrix is maintained as a strained matrix until said at least one cell-adhesive molecule has been coupled to said at least one active ~~intermediate~~ functional group of said self-assembled monolayer.
4. (Original) The method of claim 1, wherein said self-assembled monolayer comprises an alkylsilane derivative represented by  $\text{RSiX}_3$ ,  $\text{R}_2\text{SiX}_2$ , or  $\text{R}_3\text{SiX}$ , wherein X is chloride or alkoxy, and R is a carbon chain having said at least one functional group.

Amendment and Reply

Atty. Dkt. No. P-5838

Serial No.: 10/660,760

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Title: Methods of Surface Modification of a Flexible Substrate to Enhance Cell Adhesion

5. (Original) The method of claim 1, wherein said at least one functional group of said self-assembled monolayer are selected from amines, thiols, pyridyl, carboxyl, vinyl, sulfydryl, and aldehyde groups.
6. (Original) The method of claim 1, wherein said self-assembled monolayer has native exposed functional groups.
7. (Original) The method of claim 1, wherein said self-assembled monolayer has been chemically modified to have exposed functional groups.
8. (Canceled)
9. (Currently Amended) The method of claim ~~[[8,]] 1~~, wherein said activating said at least one functional group of said self-assembled monolayer comprises treating said self-assembled monolayer with a carbodiimide and a stabilizing compound to form at least one stabilized ~~active intermediate~~ activated functional group on said self-assembled monolayer.
10. (Original) The method of claim 9, wherein said carbodiimide is ethyldimethylaminopropyl-carbodiimide (EDC).
11. (Original) The method of claim 9, wherein said stabilizing compound is selected from the group consisting of N-hydroxysuccinimide (NHS), hydroxysulfosuccinimide, and hydroxybenzotriazolohydrate.
12. (Original) The method of claim 11, wherein said stabilizing compound is sulfo-NHS.
13. (Original) The method of claim 11, wherein the concentration of each of said EDC and said sulfo-NHS are between about 0.5 mg/ml and about 10 mg/ml.
14. (Original) The method of claim 13, wherein said concentrations of said EDC and said sulfo-NHS are each about 4 mg/ml.
15. (Original) The method of claim 1, further comprising adjusting the density of said self-assembled monolayer to control the density of said at least one cell-adhesive molecule.

Amendment and Reply

Atty. Dkt. No. P-5838

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16. (Original) The method of claim 1, further comprising adjusting the density of said at least one functional group on said self-assembled monolayer to control the density of subsequently bonded at least one cell-adhesive molecule.
17. (Currently Amended) A device comprising a surface, said surface comprising
- a. a flexible polymer matrix;
  - b. a mechanically self-assembled monolayer; and
  - c. at least one cell-adhesive molecule coupled to said mechanically self-assembled monolayer through at least one functional group on said self-assembled monolayer, wherein said cell-adhesive molecule is an extracellular matrix (ECM) molecule, an antibody or antigen-binding fragment thereof, or a growth factor.
18. (Original) The device of claim 17, wherein said polymer matrix comprises a polyorganosiloxane.
19. (Original) The device of claim 18, wherein said polyorganosiloxane is polydimethyl siloxane (PDMS).
20. (Original) The device of claim 17, wherein said self-assembled monolayer is an alkylsilane derivative represented by  $\text{RSiX}_3$ ,  $\text{R}_2\text{SiX}_2$ , or  $\text{R}_3\text{SiX}$ , wherein X is chloride or alkoxy, and R is a carbon chain comprising said at least one functional group.
21. (Original) The device of claim 17, wherein said at least one functional group of the self-assembled monolayer are amines, thiols, pyridyl, carboxyl, vinyl, sulfydryl, or aldehyde groups.
22. (Original) The device of claim 21, wherein said self-assembled monolayer is a chlorosilane-based oligomer or polymer.
23. (Original) The device of claim 22, wherein said self-assembled monolayer is a trichlorosilane-based oligomer or polymer.

Amendment and Reply

Atty. Dkt. No. P-5838

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24. (Canceled)
25. (Currently Amended) The device of claim ~~[[24]]~~ 17, wherein said ~~one or more polypeptides~~ cell-adhesive molecule is an extracellular matrix (ECM) molecule.
26. (Original) The device of claim 25, wherein said ECM molecule is laminin.
27. (Original) The device of claim 25, wherein said ECM molecule is fibronectin.
28. (Currently Amended) The device of claim ~~[[24]]~~ 17, wherein said ~~one or more polypeptides~~ cell-adhesive molecule is an ~~antibodies~~ antibody or antigen-binding ~~fragments~~ fragment thereof.
29. (Currently Amended) The device of claim ~~[[24]]~~ 17, wherein said ~~one or more polypeptides~~ cell-adhesive molecule is a growth factor.
30. (Original) The device of claim 17, further comprising chemical sensor particles dispersed in said flexible polymer matrix, said particles conferring chemical sensing capability.
31. (Original) The device of claim 30, wherein said chemical sensor particles are oxygen sensor particles, capable of responding to oxygen present in a solution contacting the flexible polymer matrix.
32. (Original) The device of claim 17, wherein said polymer matrix is in the form of a three-dimensional scaffold having internal surfaces to which the self-assembled monolayer is grafted and the cell-adhesive molecule is bonded.
33. (Original) The device of claim 17, wherein said polymer matrix is characterized by a strain of up to about 200% in response to an effective stress.
34. (Original) The device of claim 17, wherein said polymer matrix is characterized by a strain of up to about 100% in response to an effective stress.
35. (Original) The device of claim 34, wherein said polymer matrix is characterized by a strain of between about 40% and about 80% in response to an effective stress.

Amendment and Reply

Atty. Dkt. No. P-5838

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36. (Original) The device of claim 34, wherein said polymer matrix is characterized in that it undergoes an elastic stress-strain response in which the polymer matrix returns to approximately its original length after application and cessation of the stress.
37. (Original) The device of claim 17, which is susceptible to deformation upon application of mechanical forces such that adherent cells cultured in said device are subjected to the mechanical forces applied to and through the polymer matrix.
38. (New) A device comprising a surface, said surface comprising
- a. a flexible polymer matrix, wherein said matrix comprises chemical sensor particles dispersed in said matrix, said particles conferring chemical sensing capability;
  - b. a mechanically self-assembled monolayer; and
  - c. at least one cell-adhesive molecule coupled to said mechanically self-assembled monolayer through at least one functional group on said self-assembled monolayer.
39. (New) The device of claim 38, wherein said chemical sensor particles are oxygen sensor particles, capable of responding to oxygen present in a solution contacting the flexible polymer matrix.
40. (New) The device of claim 38, wherein said flexible polymer matrix comprises a polyorganosiloxane.
41. (New) The device of claim 40, wherein said polyorganosiloxane is polydimethyl siloxane (PDMS).
42. (New) The device of claim 38, wherein said self-assembled monolayer is an alkylsilane derivative represented by  $RSiX_3$ ,  $R_2SiX_2$ , or  $R_3SiX$ , wherein X is chloride or alkoxy, and R is a carbon chain comprising said at least one functional group.
43. (New) The device of claim 38, wherein said at least one functional group of the self-assembled monolayer are amines, thiols, pyridyl, carboxyl, vinyl, sulfydryl, or aldehyde groups.

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44. (New) The device of claim 43, wherein said self-assembled monolayer is a chlorosilane-based oligomer or polymer.
45. (New) The device of claim 44, wherein said self-assembled monolayer is a trichlorosilane-based oligomer or polymer.
46. (New) The device of claim 38, wherein said cell-adhesive molecule comprises one or more peptides or polypeptides.
47. (New) The device of claim 46, wherein said one or more polypeptides is an extracellular matrix (ECM) molecule.
48. (New) The device of claim 47, wherein said ECM molecule is laminin.
49. (New) The device of claim 47, wherein said ECM molecule is fibronectin.
50. (New) The device of claim 46, wherein said one or more polypeptides is an antibody or antigen-binding fragment thereof.
51. (New) The device of claim 46, wherein said one or more polypeptides is a growth factor.
52. (New) The device of claim 38, wherein said polymer matrix is in the form of a three-dimensional scaffold having internal surfaces to which the self-assembled monolayer is grafted and the cell-adhesive molecule is bonded.
53. (New) The device of claim 38, wherein said polymer matrix is characterized by a strain of up to about 200% in response to an effective stress.
54. (New) The device of claim 38, wherein said polymer matrix is characterized by a strain of up to about 100% in response to an effective stress.
55. (New) The device of claim 54, wherein said polymer matrix is characterized by a strain of between about 40% and about 80% in response to an effective stress.

Amendment and Reply

Atty. Dkt. No. P-5838

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Title: Methods of Surface Modification of a Flexible Substrate to Enhance Cell Adhesion

56. (New) The device of claim 54, wherein said polymer matrix is characterized in that it undergoes an elastic stress-strain response in which the polymer matrix returns to approximately its original length after application and cessation of the stress.
57. (New) The device of claim 38, which is susceptible to deformation upon application of mechanical forces such that adherent cells cultured in said device are subjected to the mechanical forces applied to and through the polymer matrix.